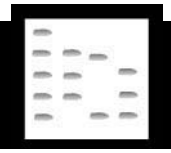
# 17. THIN LAYER (PLANAR) CHROMATOGRAPHY: DETECTION



**A. Misra**, Bareilly, India

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The following tables list detection methods and reagents suitable for detecting and identifying substances separated by thin-layer (planar) chromatography.

**Table 1.1** Methods of detection on TLC plates (aluminium oxide) by heating (Types 150/T or 60/E)

| *Substances* | *Temperature/time* | *Remarks* |
|---|---|---|
| Pesticides, e.g. aminocarb, captan, difolatan, landrin, rotenone | 200°C, 45 min | Induction of fluorescence in weakly fluorescent or nonfluorescent pesticides and amplification of natural fluorescence. There are some differences between basic and acidic aluminium oxide layers |
| $\Delta^4$-3-Ketosteroids, e.g. testosterone and *epi*-testosterone in urine | 180°C, 20 min | Pale blue induced fluorescence ($\lambda_{fl} = 440$ nm) for $\Delta^4$-3-ketosteroids, detection limit: 5 ng |
| $\Delta^4$-3-Ketosteroids, e.g. trimethylsilyl-testosterone | 180°C, 20 min or 150°C, 20 min | Conversion of $\Delta^4$-3-ketosteroids or their trimethylsilyl or acetyl derivatives in fluorescent components, whereby the detection limits were improved by 65% for the acetates. $\Delta^5$-3-keto- and $\Delta^5$-3-OH-steroids also react with the same sensitivity |
| Testosterone | 180°C, 20 min | Induced fluorescence ($\lambda_{fl} > 430$ nm, cut off filter) by thermal treatment of the chromatogram, the fluorescence increased by a factor of 2.5 by dipping in a solution of Triton X-100 − chloroform (1 + 4). Working range: 2–50 ng substance per chromatogram zone. Prewashing the layers with methanol-ammonia solution (25%) (50 + 50) increased the precision |
| Testosterone | 180°C, 20 min | Induced fluorescence and fluorescence amplification by a factor of 25 by dipping the chromatogram in a solution of Triton X-100 − chloroform (1 + 4) |
| $\Delta^4$-3-Ketosteroids, e.g. progesterone in plasma | 150°C, 20 min | Conversion of $\Delta^4$-3-ketosteroids into fluorescent derivatives ($\lambda_{fl} = 440$ nm). Relatively selective for progesterone at 150°C detection limit: 2–5 ng |

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**Table 1.2** Methods of fluorimetric detection on TLC plates (silica gel) by heating

| *Substances* | *Temperature/time* | *Remarks* |
|---|---|---|
| Essential oil components | 800–900°C | Induction of fluorescence in a special apparatus |
| Steroids, e.g. cholesterol, triolein, androsterone; sugars, e.g. fructose, glucose, ribose; amino acids, pyrimidines, purines, alkaloids | 110–150°C, 2–12 h | Conversion to fluorescent derivatives by heating |
| Alkaloids, e.g. raubasine and its metabolites in plasma, urine and bile | 120°C, 1 h | Amplification of the natural fluorescence of raubasine ($\lambda_{fl} = 482$ nm), detection limit 20 ng |
| Alkaloids, e.g. reserpine, rescinnamine | 105°C, 2 h | Induced fluorescence ($\lambda_{fl} > 500$ nm, cut off filter). Possibly formation of 3-dehydro derivatives |
| Alkaloids, e.g. reserpine, ajmaline, rescinnamine | 105°C, 2 h or 105°C, 15 h | Induction of stable fluorescence ($\lambda_{fl} > 480$ nm, cut off filter), detection limits 5–20 ng |
| Alkaloids, e.g. cocaine, ecgonine, benzoylecgonine, ecgonine methyl ester | 280°C, 8 min or 260°C, 10–30 min | Pale blue induced fluorescence ($\lambda_{fl} > 390$ nm, cut off filter), fluorescence amplification by a factor of 2 on dipping in liquid paraffin solution; detection limits: $<10$ ng |
| Alkaloids, e.g. lupanine, angustifoline, sparteine, lupinine, hydroxylupanine | 130°C, 17–35 h | Induced blue fluorescence ($\lambda_{fl} = 400$ nm), detection limits: 10 ng |
| Pesticides, e.g. dursban, azinphos-methyl, menazon, imidan, phosalone, zinophos | 200–225°C, 20–120 min | Induced fluorescence or amplification of natural fluorescence; detection limits: 10–300 ng |
| Organophosphorus pesticides, e.g. coumaphos, menazon, maretin, dursban | 200°C, 45 min | Induced fluorescence or amplification of natural fluorescence, detection limits: 1–80 ng |
| Pesticides, e.g. fuberidazol | 200°C, 45 min | Amplification of the natural fluorescence of some pesticides and bathochromic shift of the excitation and emission maxima; detection limits: 5–100 ng |
| Pesticides, e.g. coumatetralyl, methabenzthiazuron, propylisom, naptalam, thioquinox, warfarin etc. | 200°C, 45 min | Induced fluorescence ($\lambda_{fl} > 430$ nm, cut off filter); detection limits: 6–600 ng |
| Coumaphos | 200°C, 20 min | Residue analysis; induced fluorescence on heating ($\lambda_{fl} > 400$ nm); detection limit: 1 ng |
| Potasan, coumaphos, coroxon | 200°C, 20 min | Induced blue fluorescence ($\lambda_{fl} = 430$ nm or 450 nm), idenfication of the fluorescent derivatives as chlorferon or 4-methylumbelliferone |
| Coumaphos | 200°C, 20 min | Residue determination in honey, induced fluorescence ($\lambda_{fl} > 400$ nm, cut off filter); detection limit: 0.5 ng |
| Rubratoxin B | 200°C, 10 min | Induced fluorescence that can be intensified by gassing the previously heated chromatogram plates with ammonia vapours (10 min). This also alters the colour of the emitted light to pale blue |
| Glucose or methyglucosides | 135°C, 3 min or 140°C, 10 min | Induced yellow fluorescence |
| Sugar derivatives | 'Mild heating over a Bunsen burner' | No details of whether fluorescence was produced or if a carbonization reaction occurred |
| Sugars, e.g. glucose, fructose, galactose, mannose etc. | 160°C, 10 min | Production of fluorescence by heating the chromatogram after covering it with a glass plate. Sugar alcohols and $C_1$–$C_1$ bonded oligosaccharides do not react; detection limit: 10 ng |

**Table 1.2** *Continued*

| *Substances* | *Temperature/time* | *Remarks* |
|---|---|---|
| Sugars, e.g. glucose, glucosamine, fucose, raffinose, cellobiose, methylated sugars | 80 → 260°C, gradient or 200°C, 5 min | Production of fluorescence by temperature gradients (10°C/30 s) to determine the optimum heating temperature for the individual substances. Oligosaccharides require higher temperatures than monosaccharides. Detection limit: 1 nMol. The fluorescence colours are characteristic particularly for the methylated sugars |
| Lipids, e.g. $\beta$-sitosterol, geraniol, dolichol, squalene, cholesterol | 200°C, 15 min | Induced fluorescence; detection limits: $<1$ μg cholesterol |
| C-Nucleosides | Moderate heating on a hot plate | No details of whether fluorescence or carbonization was produced |
| Nomifensine and metabolites | 70°C, 2 h + $UV_{254}$ | Heating and simultaneous UV irradiation produced intense yellow fluorescence ($\lambda_{fl} > 460$ nm, cut off filter) |

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**Table 1.3** Examples of fluorimetric detection after thermal treatment of layer after chromatography

| *Substances* | *Temperature/time* | *Remarks* |
|---|---|---|
| Sugars, e.g. lactose, glucose, fructose | 120°C, 15 min | Violet fluorescence on a dark blue background |
| Sugars, e.g. lactose, glucose, fructose | 120°C, 15 min | Induced fluorescence; detection limits in nanogram range |
| Glucose, fructose | Infrared lamp or 170°C each for 3 min | Heating produced stable bluish-white fluorescence ($\lambda_{exe} = 365$ nm and $\lambda_{fl} > 400$ nm, cut off filter K 400), detection limits; 5–10 ng |
| Sugars, e.g. glucose, rhamnose, xylose etc. | 160°C, 3–4 min or infrared lamp | Induction of brilliant stable fluorescence $\lambda_{exe} = 365$ nm and $\lambda_{fl} > 400$ nm (cut off filter K 400), sugar alcohols do not fluoresce; detection limits; 5–10 ng |
| Creatine, creatinine, uric acid in urine and serum | 150°C, 3–4 min | Stable fluorescence $\lambda_{exe} = 365$ nm and $\lambda_{fl} > 400$ nm (cut off filter K 400) |
| Sugars, e.g. sucrose, ribose, xylose | 150°C, 3–4 min | Induced fluorescence $\lambda_{exe} = 365$ nm and $\lambda_{fl} > 400$ nm (cut off filter K 400) |

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**Table 2** Some substances that produce intense fluorescence when treated with ionized nitrogen after they have been chromatographed

| *Substance* | *Exposure time [s]* | *Substance* | *Exposure time [s]* |
|---|---|---|---|
| Cholesterol | 60 | Oleic acid | 180 |
| Cholesteryl pelargonate | 60 | Morphine | 180 |
| Progesterone | 60 | Codeine | 180 |
| Testosterone | 60 | Cocaine | 180 |
| Dieldrin | 60 | Dimerol | 180 |
| Tetrahydrocannabinol | 60 | Phenobarbital | 180 |
| Inositol | 60 | Chlorpromazine | 180 |
| Lauryl alcohol | 180 | d-Amphetamine sulfate | 180 |
| *n*-$C_{22}H_{46}$ | 180 | Methadone | 180 |
| Phenol | 180 | | |

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**Table 3** Reagents suitable for the recognition of functional groups

| *Functional group* | *Reagent* | *Remarks* |
|---|---|---|
| Acetylene compounds | Dicobaltoctacarbonyl | Formation of coloured complexes. After the reagent excess has been washed out, reaction with bromine vapour yields cobalt bromide, which reacts with $\alpha$-nitroso-$\beta$-naphthol to yield red chromatogram zones on an almost colourless background |
| Aldehydes | 4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald reagent) | Aldehydes yield violet chromatogram zones on a whitish-yellow background. Some alcohols form yellow to orange-coloured chromatogram zones |
| Aldehydes | 2,4-Dinitrophenylhydrazine | Formation of coloured hydrazones or osazones. It is possible to distinguish between saturated and unsaturated hydrazones using potassium hexacyanoferrate (III) |
| Aldehydes | Hydrazine sulfate + hydrochloric acid | Aromatic aldehydes yield coloured hydrazones |
| Alcohols | 4-(4-Nitrobenzyl)pyridine | Amino compounds, esters and ethers do not interfere, but phenols and acids as well as epoxides, olefins and substances containing labile halogen probably do |
| Alcohols (diols, polyols, sugars) | Lead(IV) acetate – dichlorofluorescein | Diol cleavage of vicinal diols, e.g. sugars, sugar alcohols. The lead tetraacetate consumed is no longer available to decompose the fluorescent dichlorofluorescein |
| Amines (primary) | Ninhydrin | Reddish or bluish chromatogram zones are produced, amino sugars and amino acids also react. Unexpectedly ascorbic acid also reacts |
| Amines (primary aliphatic and aromatic) | Diphenylboric anhydride + salicylaldehyde (DOOB) | Fluorescent reaction products are produced |
| Amines (primary) | *o*-Phthalaldehyde (OPA) | In the presence of mercaptoethanol *o*-phthalaldehyde reacts with primary amines and amino acids to yield fluorescent isoindole derivatives |

**Table 3** *Continued*

| *Functional group* | *Reagent* | *Remarks* |
|---|---|---|
| Amines (primary) | Trinitrobenzenesulfonic acid (TNBS) | On heating primary amines react with TNBA to yield intensely coloured Meisenheimer complexes. Amino acids also react |
| Amines (primary) | Fluorescamine | Primary aliphatic and aromatic amines yield fluorescent derivatives. Primary aromatic amines yield stable yellow-coloured derivatives that can be eluted from the TLC layer |
| Amines (primary aromatic) | Sodium nitrite + α-naphthol or Bratton-Marshall reagent | Diazotization of the primary amine followed by coupling with α-naphthol or N-(L-naphthyl)ethylenediamine. Sulfonamides also react |
| Amines (primary aromatic) | 4-(Dimethylamino)benzaldehyde + acid | Alkaloids and indole derivatives also react |
| Amines (capable of coupling) | Fast blue salt B, fast blue salt BB, fast black salt K, diazotized sulfanilic acid (Pauly's reagent), diazotized sulfanilamide or 4-nitroaniline | Intensely coloured azo dyes are produced. Catecholamines, imidazoles and phenols also react |
| Amines (primary and secondary) | 7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD chloride) | Fluorescent 4-nitrobenzofurazan derivatives are produced. Phenols and thiols also react |
| Amines (primary and secondary aromatic) | *p*-Chloranil | The reaction depends on the catalytic effect of silica gel. Monochlorobenzene as solvent for the reagent, also contributes. There is no reaction on cellulose layers |
| Amines (secondary aliphatic and alicyclic) | Sodium nitroprusside + acetaldehyde | Secondary aliphatic and alicyclic amines yield blue-coloured chromatogram zones (e.g. morpholine, diethanol amine) |
| Amines (long-chain primary, secondary and tertiary plus quaternary ammonium salts) | Cobalt(II) thiocyanate | Long-chain primary, secondary and tertiary amines and long-chain quaternary ammonium salts yield blue chromatogram zones on a pink background |
| Carboxyl groups (carboxylic acids) | Indicators, e.g. bromocresol green, bromocresol green + bromophenol blue + potassium permanganate, bromocresol purple, methyl red + bromothymol blue | Detection depends on the colour change of the indicator in acid medium. Quaternary ammonium salts give a colour change in some cases |
| Carboxyl groups (carboxylic acids) | 2,6-Dichlorophenol-indophenol (Tillmann's reagent) | Organic acids release the red undissociated acid from the blue mesomerically stabilized phenolate anion. Reductones reduce the reagent to a colourless compound |
| Carboxyl groups (carboxylic acids) | Aniline + aldose (e.g. glucose) | The action of acid causes glucose to be converted to furfural which reacts with aniline to yield a coloured product |
| Halogen derivatives | Silver nitrate, ammoniacal (Dedonder's, Tollens' or Zaffaroni's reagent) | Halogen compounds yield black chromatogram zones on a pale grey background |
| Ketones | 2,4-Dinitrophenylhydrazine | Formation of coloured hydrazones or osazones. It is possible to distinguish between saturated and unsaturated hydrazones using potassium hexacyanoferrate (III) |

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**Table 3** *Continued*

| *Functional group* | *Reagent* | *Remarks* |
|---|---|---|
| Nitro derivatives | Benzylcyanide + benzyl-trimethylammonium hydroxide | Nitro compounds, e.g. explosives, or pesticides containing nitro groups yield gray to bluish-green chromatogram zones on a brownish background |
| Peroxides | 1-Naphthol + $N^4$-ethyl-$N^{4-}$ (2-methanesulfonamidoethyl)-2-methyl-1,4-phenylenediamine (peroxide reagent) | A quinonimine dyestuff is produced on reaction with peroxides |
| Peroxides | Iron(II) sulfate + ammonium thiocyanate | Peroxides rapidly oxidize iron(II) to iron(III) ions which react to yield brown-red iron(III) thiocyanate complexes |
| Peroxides | Potassium iodide + starch | Peroxides release free iodine which forms a blue complex with the starch |
| Peroxides | N,N-Dimethyl-1,4-phenylenediamine (N,N-DPDD), N,N,N′,N′-tetra-methyl-1,4-phenylene-diamine (TPDD) | Peroxides, e.g. alkyl hydroperoxides, oxidize N,N-DPDD to Wurster's red and TPDD to Wurster's blue |
| Phenols | 7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD chloride) | Fluorescent 4-nitrobenzofuran derivatives are produced. Primary and secondary aromatic amines and thiols also react |
| Phenols (capable of coupling) | Fast blue salt B, fast blue salt BB, fast black salt K, diazotized sulfanilic acid (Pauly's reagent) diazotized sulfanilamide or 4-nitroaniline | Intensely coloured azo dyes are formed. Catecholamines, imidazoles and amines capable of coupling also react |
| Thiols, thioethers, disulfides | Sodium metaperiodate + benzidine | Substances with divalent sulfur yield white chromatogram zones on a blue background |
| Thiols | 7-Chloro-4-nitobenzo-2-oxa-1,3-diazole (NBD chloride) | Fluorescent 4-nitrobenzofuran derivatives are formed. Primary and secondary aromatic amines and phenols also react |

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*See also:* **II/Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Spray Reagents.